Oxidant and Antioxidant Status in Patients with Rheumatoid Arthritis Treated by Methotrexate

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Abstract:

Background: Rheumatoid arthritis (RA) is a debilitating, chronic multisystem disease with an unknown etiology. Recent findings indicated that increased oxidative stress and/or defective antioxidant status contribute to the etiology of RA. Cardiovascular mortality are increased in patients with rheumatoid arthritis and drugs used to treat RA may affect cardiovascular outcomes.

Objectives: To find the effect of methotrexate on the oxidant /antioxidant status in female patients with RA in comparison with female patients with RA not treated by methotrexate and the healthy control in Mosul City / Iraq.

Methods: Case control study.

Study period: Period from April 2009 to April 2010.

Subjects & Method: This study included 40 female patients, 20 of them were RA not treated by methotrexate and the other 20 patients were RA patients treated by methotrexate. Another 20 healthy women were drawn from the same population and matched for age, with the patients group and they were considered the control group.

- Serum of venous blood from all patients kept frozen at – 20°C to be analyzed thereafter for the estimation of oxidant and antioxidant parameters.

Results: A significant higher level of malondialdehyde (MDA) but non significant difference of PN2 level. Glutathione peroxidase (GSH-Px) was significantly lower but ceruloplasmin and superoxide dismutase (SOD) levels were significantly higher in RA patients without methotrexate therapy in comparison with healthy control. MDA level was significantly lower but PN2 level non significantly lower in RA patients on methotrexate therapy in comparison with RA patients without methotrexate therapy. Glutathione peroxidase (GSH-Px) is significantly higher but ceruloplasmin and SOD level is significantly lower in RA patients on methotrexate therapy in comparison with RA patients without methotrexate therapy.

Conclusion: The finding of this study suggested that methotrexate treatment for RA patients causes a reduction in oxidative stress which is cardiovascular risk factors and increase some parameters of antioxidants (especially GSH-Px) of these patients.

Key words: Rheumatoid arthritis, methotrexate, malondialdehyde, glutathione.

Introduction:

Rheumatoid Arthritis (RA) is a chronic systemic inflammatory disorder which affects women about 2.5 times more than men[1]**.** Rheumatoid arthritis (RA) is a debilitating, chronic multisystem disease with an unknown etiology. Several evidences suggest that reactive oxygen species (ROS) and oxidative stress are involved in pathogenesis of RA [2,3]. Because of impaired antioxidant system[4] it seems that RA patients are exposed to lipid peroxidation which is one of the indicators of oxidative stress [2,5] .

Most studies indicate that malondialdehyde (MDA) as a product of lipid peroxidation will increase in the serum, plasma and synovial fluid in RA[2,4,6,7]. Moreover, an inverse association between serum antioxidant levels and inflammation were reported before[8] and RA patients have lower levels of serum antioxidants, including vitamin E, vitamin C, β-carotene, selenium and zinc in comparison to healthy person [6].

Patients with RA are treated with a range of drugs lies into four classes[9]**:** including non-steroidal anti-inflammatory drugs (NSAIDs), selective cyclo-oxygenase-2 (COX-2) inhibitors, corticosteroids and disease modifying anti-rheumatic drugs (DMARDs) including antimalarials, methotrexate, tumor necrosis factor alpha (TNF-α) blockers and leflunomide. Although methotrexate was originally designed as a chemotherapy drug (in high doses), in low-doses methotrexate is a safe and well tolerated drug in the treatment of certain autoimmune diseases. Because of its efficacy and safety, low-dose methotrexate is now first-line therapy for the treatment of rheumatoid arthritis [10,11] and is used in up to 60% of patients[12].

Coronary atherosclerosis[13] and cardiovascular mortality[14] are increased in patients with rheumatoid arthritis (RA). Drugs used to treat RA may affect cardiovascular outcomes. For example, in large epidemiological studies DMARDs such as methotrexate appear to decrease cardiovascular mortality[15,16] While COX-selective drugs increase it[17]. Thus, it is important to understand the effects of drugs used to treat RA on cardiovascular risk factors. There is some information about the effects of drugs used to treat RA on cardiovascular risk factors. For example, NSAIDs and COX-2 inhibitors increase blood pressure[18], high doses of corticosteroids can cause diabetes[19], and use of antimalarials may improve glucose tolerance[20] and was associated with beneficial lipid profiles[21], however the information available is limited. The **aim** of the present study is to find the effect of methotrexate treatment on the oxidant/ antioxidant status of female patients with RA.

Patients & Methods

This study included 60 female patients, 20 of them were newly diagnosed as RA and 20 of them were RA patients treated by methotrexate in a dose ranged between 10-15 mg / week for a period ranged between 1/4-4 years. The other 20 healthy women were drawn from the same population and matched for age, with the patients group and they were considered the control group.

Five ml of venous blood were withdrawn, using a disposable syringe from the two patients groups and the control group. The blood was allowed to clot in a plain tube at room temperature and then the serum was separated by centrifugation at 3000 rpm for 10 minutes and then kept frozen at – 20°C to be analyzed thereafter for the estimation of:

1- Serum malondialdehyde (MDA) by method of Buege and Aust [22].
2- Peroxy Nitrites (PN2) activity was measured spectrophotometrically by modified method described by Vanuffelen *et al.* [23]
3- Serum glutathione peroxidase (GSH-Px) is determined spectrophotometrically by modified procedure ultizing Ellman`s reagent [24].
4- Ceruloplasmin activity was measured spectrophotometrically by modified method described by Menden *et al.*[25].
5- Superoxide dismutase (SOD) was measured by photochemical method described by Brown and Goldstein [26].

Standard statistical methods were used to determine the mean, standard deviation (SD) and the range. Paired t-test was used to compare the results of various biochemical parameters among the women in the three group. All values quoted as the mean ± SD and a P-value of <0.05 was considered to be statistically significant.

Results:

Table (1) shows a significant higher level of MDA but no significant difference between PN2 level in patients with RA without methotrexate therapy in comparison with healthy control. Glutathione was significantly lower but ceruloplasmin and SOD level were significantly higher in RA patients without methotrexate therapy in comparison with healthy control.

Table (1): Comparison between oxidant/antioxidants parameter in RA patients with no methotrexate and the healthy control

Parameter	Mean ± SD		P-Value
	RA No methotrexat	Healthy control	
MDA (□mol/L)	0.125 ± 0.069	0.081± 0.058	0.038
PN2 (□mol/L)	164.235 ± 37.350	161.572± 31.913	0.813(NS)
GSH-Px (□mol/L)	2.073 ± 1.408	4.501 ± 0.645	0.00
Ceruloplasmin(□mol/L)	479.42± 198.609	117.005 ± 57.568	0.00
SOD (□mol/L)	0.013 ± 0.002	0.006 ± 0.005	0.00

Table (2) shows that there is a significant lower level of SOD level in RA patients on methotrexate therapy in comparison with healthy control. No significant changes in the level of MDA, PN2, GSH-Px and ceruloplasmin in RA patients on methotrexate therapy in comparison with healthy control.

Table (2): Comparison between oxidant/antioxidants parameter in RA patients with methotrexate and the healthy control

Parameter	Mean ± SD		P-Value
	RA with methotrexate	Healthy control	
MDA (µmol/L)	0.075 ± 0.05	0.081± 0.058	0.777(NS)
PN2 (µmol/L)	142.84± 39.96	161.572± 31.913	0. 292(NS)
GSH-Px (µmol/L)	4.31 ± 0.60	4.501 ± 0.645	0. 084(NS)
Ceruloplasmin(µmol/L)	103. 48 ± 53.38	117.005 ± 57.568	0.405(NS)
SOD (µmol/L)	0.003 ± 0.002	0.006 ± 0.005	0.044

Table (3) demonstrates that MDA level was significantly lower but PN2 level non significantly lower in RA patients on methotrexate therapy in comparison with RA patients without methotrexate therapy. Glutathione is significantly higher but ceruloplasmin and SOD level is significantly lower in RA patients on methotrexate therapy in comparison with RA patients without methotrexate therapy.

Table (3): ***Comparison between oxidant/antioxidants parameter in RA patients with methotrexate and RA patients with no methotrexate***

Parameter	Mean ± SD		P-Value
	RA No methotrexate	RA with methotrexate	
MDA (µmol/L)	0.125 ± 0.069	0.075 ± 0.05	0.01
PN2 (µmol/L)	164.235 ± 37.350	142.84 ± 39.96	0.099(NS)
GSH-Px (µmol/L)	2.073 ± 1.408	4.31 ± 0.60	0.00
Ceruloplasmin(µmol/L)	479.42± 198.609	103. 48 ± 53.38	0.00
SOD (µmol/L)	0.013 ± 0.003	0.003 ± 0.002	0.00

Discussion:

This study found that there is a significant higher level of MDA but non significant difference between PN2 levels in patients with RA without methotrexate therapy in comparison with healthy control. Glutathione was significantly lower but ceraluplasmin and SOD level were significantly higher in RA patients without methotrexate therapy in comparison with healthy control.

The same finding was with the studies of Taysi *et al.,* [27] and Karatas *et al.,* [28]**.** In agreement with other studies [2,3,5,7] all found an oxidative stress and a low antioxidant status in serum, plasma or erythrocytes of patients with RA. Only Oliveri *et al*., [29] reported no change in lipid peroxidation in patients with RA.

There are controversial reports on erythrocyte SOD, GSHPx, and ceruloplasmin activities in patients with RA, as increased[30], unaltered[4] or decreased[31] SOD activity has been reported.

Similarly, decreased[31] which in agreement with our findings**,** or increased [4] or unaltered[30] GSHPx activity in serum or erythrocytes, also increased level of ceruloplasmin[32], which is in agreement with our findings have also been reported.

Decreased GSH-Px activity levels in patients with RA may indicate a degradation of these antioxidant enzymes by free radicals during detoxification processes and it appears that increased levels of superoxide and other radicals are not detoxified in patients with RA due to decreased efficiency of antioxidant enzymatic and non-enzymatic mechanisms, and may act as mediators of tissue damage[28]

It has been suggested that enzymatic and/or non-enzymatic antioxidant systems are impaired in RA and hence patients are exposed to oxidant stress and concluded that these changes are probably due to efforts for reducing lipid peroxidation and hence to lower tissue damage.

While Cimen *et al.,* study[30] suggested that excessive free radical production through the xanthine-xanthine oxidase system is the primary factor in rheumatoid arthritis, rather than an impaired antioxidant system and the therapeutic use of xanthine oxidase enzyme inhibitors and some antioxidants can be beneficial in this regard.

Ahmad et *al.,* [33] concluded that patients with RA have increased levels of oxidant stress, inflammation, insulin and soluble adhesion molecules. While Gambhir *et al.,* [4] found also significantly increased lipid peroxidation, measured as malondialdehyde (MDA), in the plasma of rheumatoid arthritis patients ($p < 0.01$).

The activities of erythrocyte antioxidant enzymes, superoxide dismutase and catalase remained unaltered.

However, erythrocyte glutathione and plasma ceruloplasmin levels were significantly higher in patients ($P< 0.001$) and concluded that these results suggest that increased oxidant stress present in rheumatoid arthritis may lead to compensatory changes in the levels of some antioxidants, viz. glutathione and ceruloplasmin. These changes, in turn, may provide additional protection against lipid peroxidation in rheumatoid arthritis.

Patients with rheumatoid arthritis have altered protein patterns in their serum and synovial fluid which influences the antioxidant activity of these fluids.

The raised levels of ceruloplasmin and the lower iron saturation of transferrin contribute to these differences [34], which is also in agreement with this study. Coforti *et al.,* [32] found a parallel enhancement of serum copper and ceruloplasmin in RA and they commented in view of a possible protective role of endogenous copper and/or ceruloplasmin in inflammation.

Jaswal *et al.,* [35] found a statistically significant increase in the antioxidants post treatment concentrations of antioxidants (total thiols, glutathione and vitamin C), along with a decrease in the concentrations of MDA. Recently Nourmohammadi *et al.,* [36] found also that supplementation with antioxidants yield a significantly decreased plasma MDA concentration and disease activity and significantly increase in total antioxidant capacity (TAC) level.

This study found that the use of methotrexate treatment for RA caused a significantly lower level of MDA but PN2 level non significantly lower in RA patients on methotrexate therapy in comparison with RA patients without methotrexate therapy. Glutathione is significantly higher but ceruloplasmin and SOD level is significantly lower in RA patients on methotrexate therapy in comparison with RA patients without methotrexate therapy.

According to the author's knowledge no other studies were found on the effect of methotrexate therapy on such parameters in patients with RA.

The finding of this study suggested that methotrexate treatment for RA causes a reduction in oxidative stress which is cardiovascular risk factors and increase some parameters of antioxidants of the patients.

The major findings of a study of Young Hee Rho *et al.,* [21] were that in a cross-sectional setting drugs used to treat RA including methotrexate did not have major adverse effects on cardiovascular risk factors and antimalarial use was associated with beneficial lipid profiles.

Van Ede *et al.,* [37] found that low-dose methotrexate treatment in RA patients leads to an increased plasma homocysteine level and concomitant folate supplementation with either folic or folinic acid decreases the plasma homocysteine level and consequently protects against potential cardiovascular risks but Young Hee Rho *et al.,* [21].

Found that homocysteine concentrations did not differ in patients receiving or not receiving methotrexate; this may have been the result of concurrent folate administration [37] which is common practice and routine in this cohort.

In RA, the doses utilized are much lower than oncological doses, and it is not believed that its efficacy in disease control is related to this anti-proliferative action.

Other mechanisms have been proposed, including the synthesis inhibition of toxic compounds spermine and spermidine and the extra cellular accumulation of adenosine, which has a known anti-inflammatory action mediated by the adenosine receptors[10].

In addition, it has already been demonstrated that methotrexate can suppress directly or indirectly the generation of active oxygen metabolites induced by IL-6, which in turn is produced after stimulation with TNF-α in synovial cells of RA [38] as well as in polymorphonuclear cells[39].

However, studies suggest that low doses of methotrexate induce more accentuated ROS-mediated apoptosis in lineages of lymphocyte T cells than in monocytes[40].

Conclusion: The finding of this study suggested that methotrexate treatment for RA patients causes a reduction in oxidative stress which is cardiovascular risk factors and increase some parameters of antioxidants (especially GSH-Px) of these patients.

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